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Post-Stress Combined Administration of Beta-Receptor and Glucocorticoid Antagonists as a Novel Preventive Treatment in an Animal Model of PTSD

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13. SUPPLEMENTARY NOTES

14. Many PTSD symptoms reflect pathologically enhanced memory of traumatic stress. Stress-induced secretion of brain norepinephrine and glucocorticoids (GC) activate β-receptors and GC-receptors in the amygdala, enhancing consolidation of emotional memories. We hypothesized that giving a β-antagonist plus a GC-antagonist immediately after stress might decrease the strength of those associations, and prevent the emergence of PTSD. The goals of this work were to 1) validate a reliable model of PTSD amenable to testing acute drug treatment; 2) establish a reliable and valid test battery of PTSD-like behaviors in rats, including fear conditioning and extinction; and 3) test the combined drug treatment using the established model. Although we developed a reliable test battery for PTSD-like behavior, it proved more challenging to establish a consistent, reliable and valid model inducing PTSD-like behaviors. The proposed massed footshock model, derived from the literature, failed to affect the most relevant behavioral measures, and confounded fear conditioning. A modified Single Prolonged Stress model, also from the literature, was more promising but had only modest effects. Early results using a new combined Chronic + Acute Prolonged Stress (CAPS) model, incorporating both chronic and acute features of stress often associated with PTSD, were very promising, but mixed results were obtained with the acute drug interventions. Propranolol had no effect, and mifepristone had only a modest effect on extinction. Nonetheless, this project has been very productive, as we will continue to use this model in other ongoing studies. If PTSD is indeed best modeled by a combined chronic + acute stress, acute drug intervention may not be a feasible strategy.

15. SUBJECT TERMS
chronic and acute stress, PTSD, open field test, social interaction test, fear conditioning, extinction, beta-adrenergic receptors, glucocorticoids, propranolol, mifepristone, rats

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Table of Contents

Project W81XWH-08-1-0202 (CDMRP PTSD Concept Award PT073760)
Post-Stress Combined Administration of Beta-Receptor and Glucocorticoid Antagonists as a Novel Preventive Treatment in an Animal Model of PTSD
Final Report

A. Introduction

Many symptoms of PTSD can be considered as examples of an overly enhanced and inappropriately persistent memory of the traumatic experience. The acute response to stress includes sympathoadrenal activation, with secretion of glucocorticoid hormones and plasma catecholamines, and release of the stress-responsive neurotransmitter, norepinephrine (NE), throughout the brain. NE facilitates the physiological, behavioral and cognitive responses to stress. The literature also suggests that NE and glucocorticoids act on β-adrenergic receptors and glucocorticoid receptors in the amygdala to enhance conditioned fear and anxiety, and to strengthen emotional memories of intensely stressful events during the phase of memory consolidation immediately following the experience. Thus, the hypothesis behind this project was that giving a combination of a β-adrenergic antagonist plus a glucocorticoid receptor antagonist immediately after a traumatic experience might decrease the strength of the abnormally enhanced fear-provoking memories, and reduce PTSD-like symptoms that emerge over time. To test this hypothesis, we proposed using a published rat model of PTSD, acute massed footshock (MFS) (Stam, 2007), and then employ a battery of tests designed to assess PTSD-like behavioral changes in rats, including social withdrawal on the social interaction test, generalized anxiety and stress-sensitization on the elevated plus-maze, enhanced fear conditioning, and attenuated extinction of cue-conditioned fear. One reason that we initially selected the MFS model was that the acute temporal nature of this stressor made it amenable to testing acute drug treatment in the immediate post-stress period.

In the first year of funding, we made progress toward many of our goals, but we also ran into some significant impediments. We successfully established a reliable and effective behavioral test battery to assess key PTSD-like behavioral changes that emerge over time after a traumatic experience in rats. Some of those tests were already in use in our lab, but had to be modified and adjusted to suit the time frame required by the design of these experiments. Others we had to develop, set up, and validate for the first time in our lab, including the fear conditioning and extinction paradigm, which we achieved successfully. However, after extensive testing and modification of the MFS model and several variants thereof, we concluded for many reasons that it is neither a valid nor useful model of relevance to PTSD. Thus, we formulated a viable plan to replace MFS with a different model, again taken from the literature. That is where the previous progress report leaves off. As this current document is a final report, a summary of that material from the first year will be provided below, but in a condensed form. The remainder of this report then will address our activities and progress in the six months following the last report, and the 4 months of no-cost extension allowed through April, 2010.

B. Body

B.1. Summary of work done through July, 2009 (presented in the prior Annual Report)

Attempts to validate the massed footshock model

In the first study, 3 groups of rats were exposed to a single session of massed footshock (MFS; 10 x 5 sec scrambled footshocks, 1.25 mA, delivered at varying intervals for 15 min) or served as unstressed controls. They were then tested over 4 days, beginning at either 7 or 14 days after MFS exposure, in the Open Field Test (OFT), the Social Interaction (SI) test, and the Elevated Plus Maze (EPM). The only significant effect was a decrease in locomotor activity on the OFT at 7 days, but not 14 days post-MFS (Figure 1). There were no effects on the EPM or SI test, contradicting the long-lasting changes reported after MFS in the literature (Stam 2007).

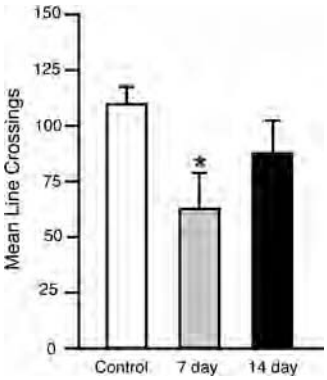

Figure 1. Rats exposed to a single MFS session exhibited reduced locomotion in the open field test 7 days after the MFS session (*p < 0.05 compared to controls). By 14 days, locomotion in the OFT had returned to control levels. Data expressed as mean number of line crossings in 5 min (mean ± SEM, n = 14 per group).

Given the minimal effect of single MFS exposure on behavior only on the OFT, and the absence of any effect on the more critical measures for modeling components of PTSD, we next attempted to modify the MFS procedure to enhance the impact, yet retain the timing needed to test the efficacy of an acute pharmacological intervention. Thus, instead of a single MFS exposure, we used three MFS sessions separated by one week. The reduced exploratory behavior seen in the OFT in the first experiment was replicated, and there was a reduction in Social Interaction, but again there was no effect on the EPM (data not shown, see annual report, July, 2009). The decrease in social interaction was promising, but this also was not replicated consistently in subsequent experiments (see below).

In the next experiment, we modified and simplified our behavioral test procedures to reveal any subtle effects of the 3-MFS treatment compared to controls. As in the previous study, there was a significant effect of the 3-MFS treatment on locomotion in the OFT, and on social interaction in the SI test, but we continued to be unsatisfied with the lack of effect on the EPM. Also, there was not the extended duration of effect we had hoped to achieve. Still, this limited success offered justification to try an initial test of the drug treatment regimen originally proposed, but with the following modifications: the stress treatment would include 3 weekly MFS sessions, and the time for testing was compressed to 3-5 days after the last MFS session.

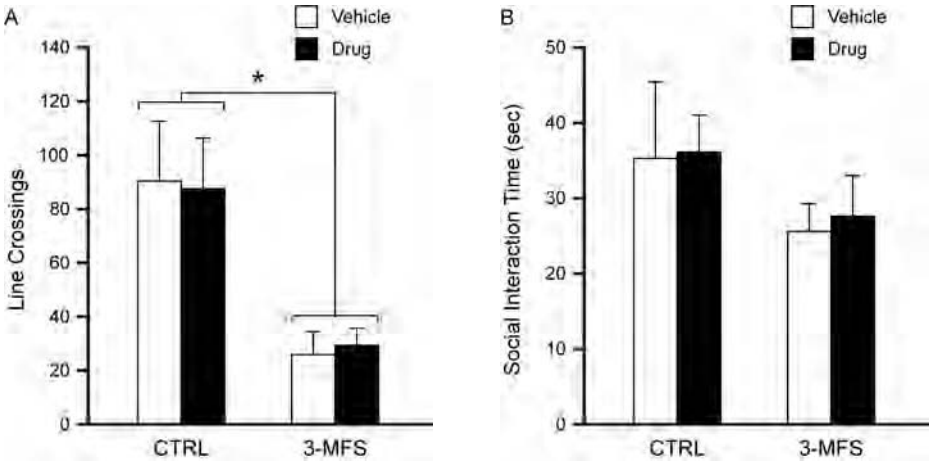

Figure 2. Rats tested 4 days after the last of three MFS sessions exhibited reduced locomotion in the open field test (A) but in this study, social interaction was unaffected (B). Combined treatment with the β-receptor antagonist, propranolol (10 mg/kg) and glucocorticoid-receptor antagonist, mifepristone (25 mg/kg) given immediately after each MFS or control session, had no effect on baseline behavior in either test, nor on the reduction in OFT behavior seen after MFS exposure. *p < 0.01 compared to controls; mean ± SEM, n = 7-9 per group.

In this first test of the combined drug intervention intended to attenuate consolidation of the aversive consequences of MFS exposure, we focused on giving the drug treatment immediately after each MFS exposure, as proposed in Task 3 of the original SOW. The reason was that post-stress treatment would be most relevant to the consolidation process, and would also be the most likely mode of any potential treatment in the field, after exposure to a traumatic event. Rats were assigned to MFS or control groups. The MFS treatment was given in 3 weekly sessions. Immediately after each MFS or control session, rats received injections of propranolol (10 mg/kg in a volume of 3 ml/kg, i.p.) and mifepristone (25 mg/kg in a volume of 1 ml/kg, i.p.) or comparable injections of saline vehicle, and returned to the housing facility 1 hr after injections. Behavioral testing took place on day 3 (OFT), day 4 (SI) and day 5 (EPM) after the last session. Data were collected from two independent cohorts of rats. The reduction in exploration on the OFT was replicated (Figure 2A), but this time there was no significant effect of the 3-MFS treatment on social interaction (Figure 2B), and again there was no effect on EPM. Moreover, there was no effect of drug treatment on any measure (Figure 2). However, given the lack of MFS effect on either SI or EPM, the lack of drug effect was largely uninformative. Thus, at this point, we were unsatisfied with MFS. Given in a single session or in 3 sessions, it appeared to be neither a replicable nor reliable model of lasting behavioral changes relevant to PTSD.

Procedure for fear conditioning and extinction

A critical dimension of PTSD is the sensitization of fear conditioning, and failure to extinguish conditioned fear. This was the only behavioral test procedure in the proposal that had not yet been established in our lab. Thus, we purchased equipment for fear conditioning and extinction (using a different funding source, as this technique would be used more generally in our research program). As described in more detail in the annual report, we established initial parameters and test conditions that generated a reliable conditioned-fear response, and a quantifiable process of extinction (Figure 3). Rats were presented initially with 2 pairings of a 20

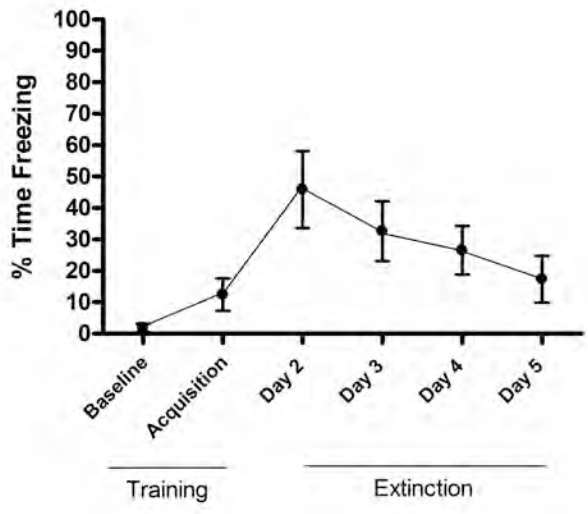

Figure 3. The newly-established fear conditioning procedure generated low baseline freezing, increased freezing in response to the tone with acquisition of tone-shock conditioning, a greater increase, to nearly 50% freezing with consolidation and retention on day 2, followed by a gradual decrease over days 2-5 with extinction. Each point represents the mean ± SEM of the 3 tones presented each day (n=5 rats).

sec tone (10 kHz, 75 dB) that co-terminated with a footshock (0.5 sec, 0.7 mA) delivered through a grid floor. On day 2, rats were then tested for retention, and the dependent measure was freezing in response to presentation of the tone alone, with no shock delivered. This also constituted the first extinction trial, with 3 extinction trials (ITI 90-120 sec.) on each of days 3-5. Freezing was recorded and analyzed from an activity histogram that plotted the number of video frames on the y-axis and the motion index (a videometric measure of the number of pixels that change from frame to frame) on the x-axis. A freezing threshold was determined for each rat from activity recorded over a 5 min habituation period. Freezing, i.e., activity below threshold, was then quantified and expressed as a percentage (*m*) of time during each 20-sec tone.

Confounding effects of MFS on fear conditioning and extinction

A valid fear-conditioning protocol was key to establishing the validity of any proposed stress model, and any treatment strategy. Thus, rats were exposed to a single MFS or control session, and 5 days later, fear conditioning and extinction were conducted as above. The behavioral response during conditioning and extinction was similar to that described above. However, although both groups showed acquisition, MFS exposure ***impaired*** fear conditioning (Figure 4), opposite to the effect predicted. We determined from the conditioning literature that the pre-exposure to footshock in the MFS was likely to have precluded its effectiveness as an unconditioned stimulus for fear conditioning. This confound, along with the inconsistent effects obtained on the SI and EPM tests (see above), made MFS an unsuitable model for this project.

Figure 4. Effects of a single MFS exposure on fear conditioning and extinction. Both MFS and control rats showed low baseline freezing, and both groups exhibited an increase in freezing in response to the tone after it was paired with footshock. However, acquisition of fear-conditioning was impaired in the MFS-pre-treated rats (mean ± SEM; n=4-5 per group).

B2. Work Done Since the last progress report, 08/09-04/10

Refine and optimize the fear-conditioning and extinction paradigm

The first purpose in the next phase of this project was to continue to refine the basic fear conditioning and extinction paradigm we had just established in the previous period, primarily to optimize the sensitivity to detect changes in both directions, and especially in the measure of extinction. First, we varied the number and intensity of tone-shock pairings delivered on the training day (tested 2-6 pairings, with shock always 0.5 sec in duration presented at the end of the 20 sec tone, and intensity ranging from 0.70-1.0 mA). We then tested the number and timing of extinction trials. We first tested blocks of 3 trials per day given over 3-5 days, the protocol we had initially started with. We then tested compressing the extinction process into one series of 10-20 trials in a single day, with retention of extinction assessed the following day. Using this overall strategy, by varying several different parameters in small experiments, with separate cohorts of 3-4 rats each, we ultimately decided on a standard procedure that we felt was most sensitive, most convenient, and also most conducive to being incorporated into the timing and experimental design of this project (see Figure 5). The final procedure entailed delivering a series of 4 tone-shock pairings on the conditioning day, with 0.70 mA shock intensity. This resulted in a level of freezing of ~50-60% on the first extinction trial (which represents a measure of retention of the conditioned association), allowing both increases and decreases to be detected. Extinction is tested 72 hr after conditioning, allowing for any potential intervening manipulations or treatments. For extinction, we give 10 trials of tone alone in one day, spaced on average 2 min apart. This is neither too few nor too many trials (which could result in over-training), as we have found that asymptotic extinction is typically achieved after 7-8 trials, but with a partial return of the conditioned response on the first retention trial the next day. And on the last day, we use 10 trials for extinction retention, identical to the procedure for extinction.

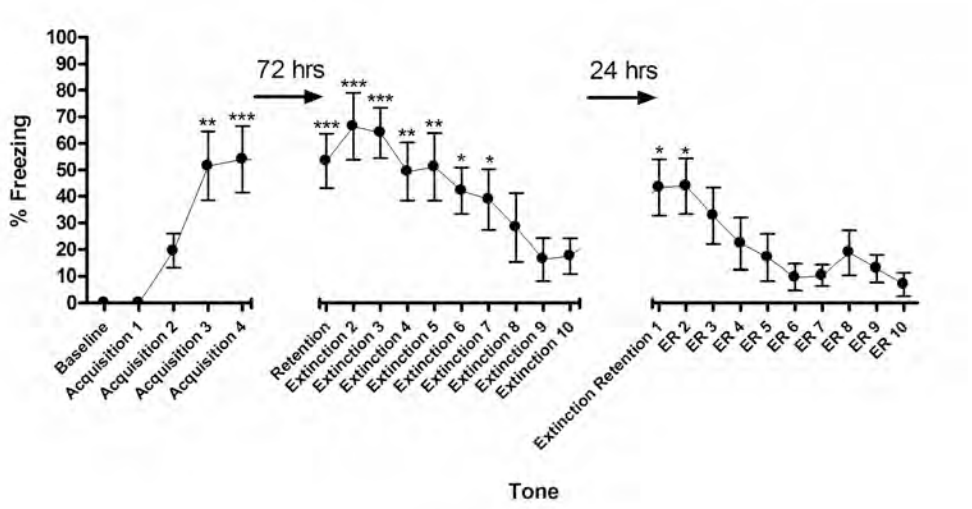

Figure 5. Fear conditioning and extinction demonstrated with our refined protocol. Naive rats (n=8) were conditioned with 4 pairings of tone (10kHz, 75 dB, 20 sec) co-terminating with footshock (0.7mA, 0.5 sec). 72 hrs later, extinction consisted of 10 presentations of tone alone, and 24 hrs later, they were tested for extinction retention, again with 10 tones. During extinction, the rats displayed an initial retention of conditioned fear, gradually decreased to a level minimum by tone 9. Upon testing for extinction retention, a partial fear response returned, and quickly declined over 5-6 trials. *p<0.05, **p<0.01, ***p<0.001 compared to pre-tone baseline.

Test and adjust the SPS model – new task 1 in revised SOW

In parallel with refining the fear conditioning and extinction procedure, we also began to test the new Single Prolonged Stress (SPS) model, as described in the revised SOW. This is another rodent model of PTSD that has been reported in the literature to have lasting behavioral effects (Khan & Liberzon, 2004; Wang et al., 2008). The original published procedure involved sequential application of three acute stressors in a single session: immobilization, forced swim, and then anesthesia were used as stressors (Wang et al., 2008,). However, we chose not to use anesthesia as a stressor because we felt that it may present a confound for testing an acute post-stress drug intervention to interfere with consolidation and sensitization of conditioned fear. Thus, we modified the published procedure slightly, using another acute stressor, social defeat stress, in place of anesthesia. The 1-hr SPS treatment thus entailed: 30 min of immobilization stress, followed by 20 min social defeat (a single, brief encounter with a resident rat, resulting in social submission by the test animal, followed by 20 min of continued exposure to the aggressor rat under a protective wire mesh cage to prevent any further physical contact), followed immediately by 10 min swim stress. This kept the duration of the entire procedure to a single 1 hr session, consistent with the aims of the original proposal, retaining the feasibility of delivering a single acute drug treatment immediately after the end of the session. SPS treatment was applied to two groups of rats (n=6 each), with two independent control groups (n=4 each) used for comparison. One group each of the stressed and control rats were then tested beginning on day 2 after SPS with the OFT, followed by the SI test on day 3. The other groups were tested beginning on day 5 with the OFT and on Day 6 with the SI test, comparable to the testing procedure we had used for the MFS previously. Once again, the only effect of SPS was on the OFT, with a reduced level of exploration in the Center region (Figure 6A - Center Time is a more sensitive and direct measure of anxiety than Line Crossings, which is more a measure of locomotion). SPS, like MFS, had no consistent effect on SI in this study, and no time-dependent effects were evident over 2-5 days. Also, we conducted a pilot study testing potential effects on extinction in a separate group of rats 5 days after SPS, using the newly standardized protocol. SPS had a modest, non-significant attenuating effect on extinction

(Figure 6B). In sum, the SPS model was more promising than the MFS model. Effects were in the right direction, albeit of only moderate impact, and it proved amenable to use with the fear conditioning and extinction protocol (unlike MFS). Still, we had hoped for a more robust effect, to be sensitive to therapeutic intervention.

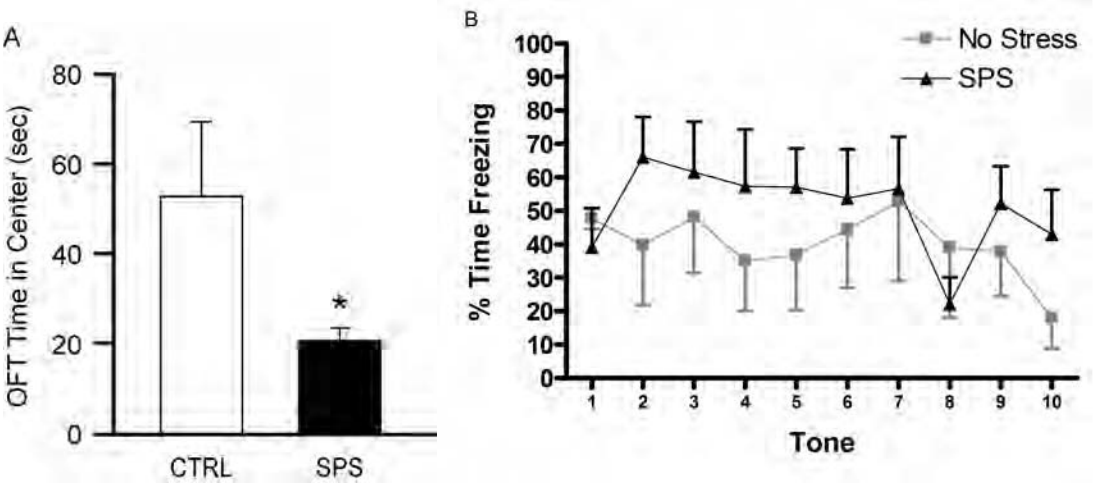

Figure 6. Effects of acute SPS on anxiety-like behavior, measured by Center Time in the OFT (A), and on extinction of conditioned fear (B), tested in separate groups of rats, 5 days after SPS treatment. SPS induced a near-significant anxiety-like decrease in center exploration on the OFT (*p=0.058), and a moderate, non-significant attenuation of extinction learning following fear conditioning (n=4-6/group in both tests).

A new mindset is required: Development of a Chronic + Acute Prolonged Stress (CAPS) model

At this point, we were getting discouraged. After our experience with MFS, we were not optimistic about chasing after yet another marginal effect, nor another model of questionable validity, based solely on the reports of a single research group in the literature. Thus, we relied on our own past experience, and we were also able to cross-fertilize this project with work we were doing in parallel on our sub-project within the large CDMRP-funded STRONGSTAR PTSD Research Consortium, focusing on factors determining vulnerability to PTSD with chronic stress treatment. A chronic stress model we had used in the past, which had produced robust and relevant effects in previous projects, was chronic intermittent cold stress (CIC). This is particularly relevant to this project, because we have shown sensitization of the brain NE system after CIC (Pardon et al., 2003, Ma et al., 2005). We have also shown that CIC produces a cognitive deficit in reversal learning, related to hypoactivity in prefrontal cortex (Lapiz-Blum, 2009), which is relevant to both PTSD and co-morbid depression. In fact, extinction learning is a very specialized form of cognitive flexibility that is also thought to require the integrity of prefrontal cortex, and in a pilot study for another related project, we have shown that CIC stress treatment itself produced a modest attenuation of extinction learning after fear conditioning (Figure 7). The CIC stress procedure is quite simple and very innocuous. Rats are placed, in their home cage with food, water and bedding, into a cold room at 4 °C for 6 hours each day for 14 days. This is a very low-level metabolic stressor - the rats generally sleep, even while mounting a stable physiological stress response. Moreover, the chronic treatment results in increased release of NE in the brain, increased sensitivity of NE receptors in the brain, and sensitization of the HPA hormonal response to subsequent acute stress (Ma et al., 2005). However, CIC lacks the acute quality that is necessary to allow the acute drug treatment required for this project. Also, the nature of this stimulus is intuitively dissimilar to the kinds of stressors that often lead to PTSD. So we began looking for a way to adapt this procedure to better suit the needs of this project.

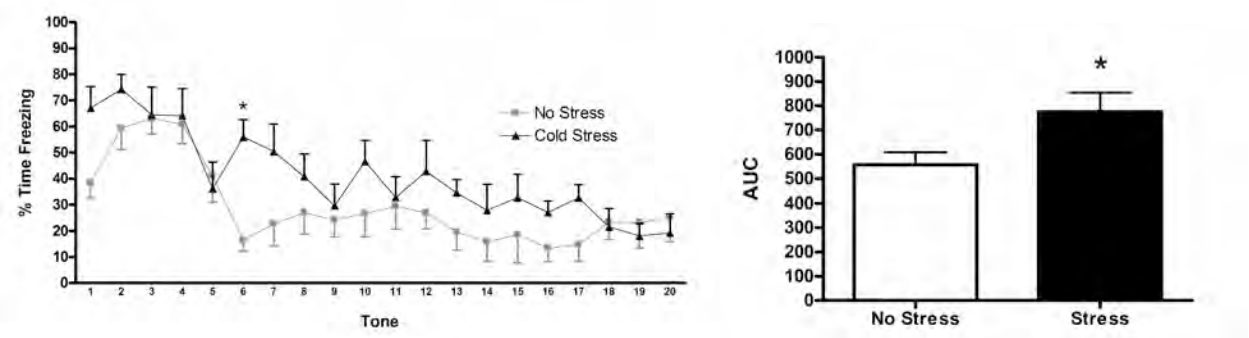

Figure 7. Extinction is attenuated after CIC stress, (data from another project). Left panel: freezing across extinction trials. Right panel: comparison of the area under the curve (AUC) for the two extinction graphs. CIC stress significantly attenuated extinction learning (main effect of stress, $p<0.05$ by ANOVA in the left panel, *$p<0.05$ comparing AUC in the right panel). Whereas freezing behavior in the control group had reached a minimum by tone 6, the CIC-stressed group remained elevated longer (n=8/group).

In subsequent consultation with our military and medical colleagues in the STRONGSTAR Consortium, it occurred to us that the chronic, low-level and tonic cold stress stimulus is reminiscent of the constant and tonic state of chronic stress that accompanies deployment to a combat zone. Further, this background state of chronic stress may then "prime" an individual to respond more robustly to a subsequent acute, traumatic stressful event superimposed on this "state". Thus, combining the two treatments, Chronic Intermittent Cold (CIC) stress, applied over a two week period, followed by the acute Single Prolonged Stress (SPS), applied in a single intense 1-hour session, as described above, at the end of that period, may represent a potentially valid and effective model of stress related to PTSD. *We feel that such a combined model has a degree of face validity, specifically for studying PTSD, because combat deployment creates a context of chronic stress, but within this context, it is often a single traumatic event that triggers the development of PTSD and provides the explicit memories and cues associated with symptoms.* We called this new protocol Chronic + Acute Prolonged Stress (CAPS). As a result of our efforts on this Concept Award project, we have begun to use the CAPS treatment as the model of adult traumatic stress in our other project, within the STRONGSTAR Consortium, to test the mechanisms of prenatal stress as a factor in vulnerability to develop PTSD upon adult exposure to traumatic stress. Thus, although the duration of this Concept Award was perhaps too short to fully capitalize on the efforts aimed at developing a suitable rat model of PTSD, it was nonetheless a very productive source of synergism and cross-fertilization for the larger, more sustained STRONGSTAR project.

Test of the acute drug treatments given after the SPS component of CAPS – new task 2

However, by this point we were well into the no-cost extension period of this project. While the CAPS model was promising, and was already being used with some early signs of success in our STRONGSTAR project, it is still in the early stages of development, and we really only had one last chance to test the acute drug interventions that were the focus of this project in the first place. The nature of the CAPS model was more complex than the acute models we had tested earlier, with both a chronic component and an acute component, but as this seems a more realistic model of the nature of deployment- and combat-related stress, it also seemed worthwhile to test the drug interventions as best we could. Thus, we decided to administer the drugs once acutely, as originally planned, immediately after the single acute SPS stress treatment at the end of the 2-week chronic CIC stress period. In addition, an abstract presented at the Military Research Forum meeting in Kansas City in 2009 had suggested that while both mifepristone and propranolol alone interfered with consolidation of fear conditioning

memory, they actually seemed to negate each others effect when given in combination, a somewhat counter-intuitive observation, but one that we considered a potential confound in our study. Thus, because both of these drugs alone had been shown previously to have effects on consolidation, we decided to give each drug alone, not in combination, after the SPS treatment.

Rats were either exposed to CAPS treatment (14 days of CIC exposure, followed on day 15 by a single SPS session) or served as unstressed controls. These groups were divided into sub-groups based on the drug given immediately after the SPS session or corresponding control period. Separate groups of stressed and unstressed control rats received the β-adrenergic receptor antagonist, propranolol (10 mg/kg in 3 ml/kg, i.p.) or its saline vehicle; or the glucocorticoid receptor antagonist, mifepristone (25 mg/kg in 0.6 ml/kg, i.p.) or its vehicle, 80% ethanol. We also included CAPS-stressed and unstressed control groups with no drug or vehicle injections. All groups had 7-10 rats, and testing focused on the anxiety measure we had detected most reliably using the OFT. Because of the extended time required for adding fear-conditioning and extinction to this already complex design, we could only pilot that measure for the purpose of procedural modifications for future use, but not for a conclusive assessment.

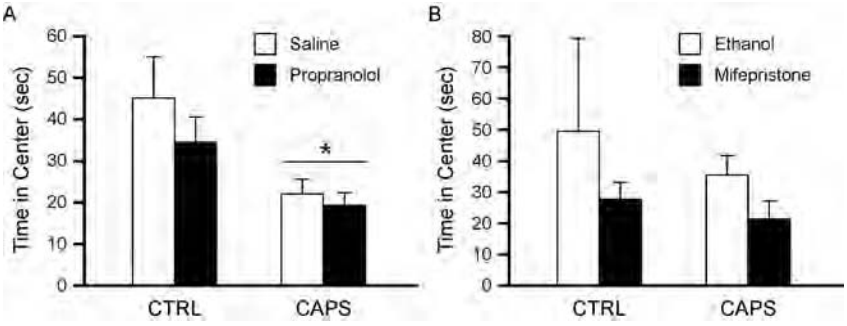

Figure 8. Lack of effect of propranolol (A) or mifepristone (B) given after the SPS session of the CAPS treatment on Center Time in the OFT (*p<0.05 for the main effect of CAPS compared to un-stressed controls; n=7-10).

No effects were seen for either drug treatment on the anxiogenic effect of CAPS on Center Time in the OFT (Figure 8). Note that in the mifepristone study, due to variability in the control group, the CAPS effect was not significant. There was also no effect of propranolol on the measures of fear conditioning and extinction after CAPS. However, in the mifepristone study, a significant interaction (p<0.05) showed that the effect of CAPS, attenuating extinction in the vehicle-treated group, was reduced by mifepristone (Figure 9). Although the effect was still modest, this was the only promising result we obtained with these drugs.

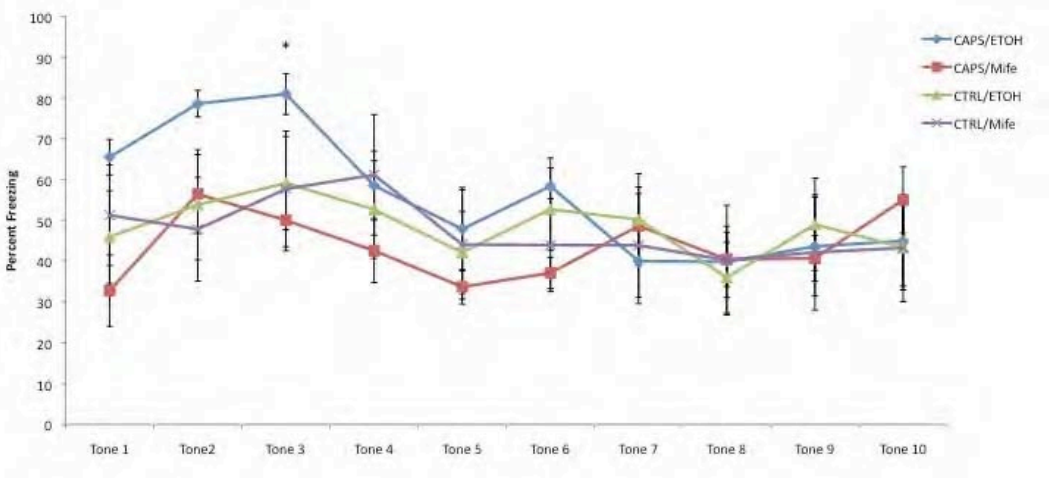

Figure 9. Mifepristone modestly reduced the magnitude of the CAPS effect, which attenuated extinction learning in the early trials of the extinction sequence (*p<0.05 CAPS-vehicle compared to CAPS-mifepristone, trial 3 only, with a significant interaction; n=7-10).

C. Key Research Accomplishments - Entire Project Period

-Established a reliable and effective behavioral test battery to assess key PTSD-like behavioral changes that emerge over time after a traumatic experience in rats

-Established and refined a reliable fear conditioning and extinction protocol that is sensitive to both enhanced and attenuated conditioning and extinction learning

-Established a drug treatment regimen that is feasible in the proposed paradigm, does not interfere with behavioral testing, and does not elicit non-specific effects in control animals

-Tested extensively the MFS model and the 3-MFS variant thereof, and concluded that it is neither a valid nor useful model of relevance to PTSD

-Tested a modified SPS model, and determined that it was more promising than the MFS model in that it had modest effects on extinction as well as anxiogenic effects on the OFT, but it was not robust, reliable, nor consistent enough to be sensitive to potential therapeutic interventions

-Relying on our past experience with chronic stress, on our previous demonstrations of effects on measures of cognitive flexibility in prefrontal cortex and NE sensitization, and on results being generated in our other ongoing research projects, we formulated a plan, and began early-stage testing of a more credible model of PTSD, the Chronic + Acute Prolonged Stress (CAPS) model. This combines a chronic state of low-level background stress, induced by 2-weeks cold exposure, with the single intense acute SPS treatment, given at the end of the two weeks. In pilot results, CAPS induced anxiety responses on the OFT and modest attenuation of extinction.

-Finally, we tested the acute drug interventions, giving the β-adrenergic antagonist, propranolol and/or the glucocorticoid antagonist mifepristone, together or alone, following the SPS component of the CAPS treatment. Propranolol had no effect, and neither drug influenced the anxiogenic response, but mifepristone modestly reduced the effect of CAPS on extinction.

D.1 Reportable Outcomes – entire project period

1. Meeting abstract – for both a poster presentation and a talk:

Morilak, DA, Joshi, A, Rodriguez, G (2009) Developing a rat model of delayed behavioral stress reactivity in PTSD suitable to investigate potential pharmacologic interventions. Congressionally Directed Medical Research Programs Military Health Research Forum, Kansas City, MO, Aug 31-Sept 3, 2009.

2. Meeting abstract – for poster presentation:

Joshi, A, Rodriguez, RA, Morilak DA (2009) Delayed stress reactivity after footshock: A rat model for PTSD. Soc Neurosci Abstr 35 Online Program no. 841.11.

3. Meeting abstract - for poster presentation:

Green MK, Joshi A, Frazer A, Strong R, Morilak DA. Increased stress-reactivity and impaired fear extinction after stress: developing a rat model of PTSD. Neurobiology of Stress Workshop, Boulder, CO, June 15-18, 2010.

4. Meeting abstract - for poster presentation:

Green MK, Joshi A, Frazer A, Strong R, Morilak DA. Prenatal stress increases stress-reactivity and impairs fear extinction after adult stress: a model of PTSD. Soc Neurosci Abstr 35 Online Program, in press.

D.2 Personnel receiving pay from this research effort – entire project period

Senior investigators

David Morilak, Ph.D., Principal Investigator
Milena Girotti, Ph.D., Post-Doctoral fellow

Research staff

Rami Weaver, Research Asst.
Gus Rodriguez, Research Asst
Julianne Doyen, Research Asst
Ashley Furr, Research Asst
Ankur Joshi, Research Asst.

E. Conclusions and plans

Perhaps the most important conclusion to draw from these results might be that the stress of deployment and combat most likely to lead to PTSD is best modeled as a chronic, tonic background "state of stress" combined with an acute, intense traumatic stressor superimposed on that chronic state. As such, it may be difficult to treat or prevent the occurrence of PTSD with a single acute drug intervention. The data and procedures developed in this project will continue to inform and contribute to the continued progress of our active STRONGSTAR study.

F. References

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Pardon, M.-C., Ma, S. and Morilak, D.A. (2003). Chronic cold stress sensitizes brain noradrenergic reactivity and noradrenergic facilitation of the HPA stress response in Wistar Kyoto rats, *Brain Research,* 971: 55-65.

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Wang W, Liu Y, Zheng H, Wang HN, et al. (2008). A modified single-prolonged stress model for post-traumatic stress disorder. *Neuroscience Letters* 441, 237-241.